Biomedical Research 2017; 28 (22): 9998-10004 *ISSN 0970-938X*
www.biomedres.info

Utility of single nucleotide polymorphism status of vascular endothelial growth factor in predicting long-term effects of bevacizumab in treating metastatic colorectal cancer.

Wei Wang*, Lirong Zhao, Jing Wu, Fen Feng, Yunchang Chen

Cancer Center, the First People's Hospital of Foshan, Foshan, PR China

Abstract

The aim of the current study was to investigate the relationship between Single Nucleotide Polymorphisms (SNPs) of Vascular Endothelial Growth Factor (VEGF) in peripheral blood vessels and the prognosis of patients with Metastatic Colorectal Cancer (MCC). Peripheral blood samples of a total of 60 MCC patients treated in our hospital from 2012 to 2015 were tested for nine VEGF SNPs by MassARRAY, including -2578C>A, -460T>C, -1455T>C, -1154G>A, -634G>C, -398G>A, -497T>C, -2455>-T, and -936C>T. For the 60 MCC patients, the SNP variability for VEGF was similar to that in the NCBI database; the Overall Survival (OS) of patients homozygous for SNP-497TT was worse than that of patients with other SNPs (P=0.02). Among the patients treated with Bevacizumab (BEV), the OS for three SNP-497 genotypes showed significance (P=0.01), the OS of patients homozygous for SNP-398AA and SNP-2455CC was better than that of patients with the other SNPs (P=0.02, and P=0.01, respectively). The SNP status of VEGF in peripheral blood may be related to BEV treatment and long-term chemotherapeutic efficacies.

Keywords: Metastatic colorectal cancer, Bevacizumab, Vascular endothelial growth factor, Single nucleotide polymorphism.

Accepted on October 18, 2017

Introduction

The incidence and mortality of colorectal cancer in China are rising [1], and the median survival for Metastatic Colorectal Cancer (MCC) is about 30 months, even with more expensive targeted chemotherapies, such as those with bevacizumab (BEV; a vascular endothelial growth factor antibody) or cetuximab (an anti-epidermal growth factor receptor monoclonal antibody). Many Chinese patients cannot afford the cost of targeted therapy. Improving the curative effects and prolonging the survival period under limited social and economic resources has become an important research subject. Simple clinical prognostic factors such as gender, metastatic location, or physical condition are not always diagnostically valuable; thus, identification of colorectal cancer patients suitable for targeted therapies on the basis of their molecular characteristics has become a pertinent topic of research in recent years. Genome research is making true individualized treatment possible. For example, KRAS gene mutation detection has become the gold standard for predicting the efficacy of cetuximab in the treatment of colorectal cancer. However, there are currently no predictive or prognostic biomarkers in clinical practice for the use of the targeted therapeutic drug BEV. BEV causes cardiovascular and cerebrovascular injury as well as other undesirable side effects [2,3]; thus, it is clinically important to determine factors that can predict BEV effectiveness and toxicity so as to provide benefit to the patients and avoid unnecessary economic loss and toxicity. With regards to predicting the efficacy of BEV, the first concern was focused on the expression of Vascular Endothelial Growth Factor (VEGF) in tumor tissues and its concentration in peripheral blood. Retrospective analysis of four large phase III clinical trials (AVF 2107g, AVAiL, AVOREN, and E4599) [4] confirmed that neither VEGF expression in tumor tissues nor baseline plasma level prior to treatment is correlated with the efficacy of BEV. Analysis of related tumor genes and proteins [5-7] revealed that deletion of the *PTEN* gene and expression of the VEGF-A/D protein may be related to BEV efficacy, but the results of different studies are not consistent. After all, the target of BEV is VEGF in peripheral blood, and tumor tissue gene expression may have no promotive effect on its efficacy and toxicity. Recent studies have focused on determining efficacy-predicting factors present in peripheral blood, and a phase II clinical trial conducted by Willett et al. [8] has shown that the concentration of VEGF in the plasma of 32 rectal cancer patients receiving BEV was increased compared to that before treatment, and it may be related to the reduction of the primary tumor and lymph nodes to a certain extent. It is also possible that pre-and post-treatment plasma VEGF concentrations are more related to the efficacy of BEV. Thus, determination of VEGF concentrations in peripheral blood before, during, and after an

adequate treatment course is necessary to determine whether VEGF can be used to predict the efficacy of BEV. These hypotheses require further prospective studies for confirmation, however, there is no standardized process to detect VEGF in peripheral blood, and consequently, clinical monitoring of VEGF concentration in peripheral blood is not yet operational. The *VEGF* gene is subject to several Single Nucleotide Polymorphisms (SNPs). Two retrospective analyses evaluating MCC patients treated with BEV combined with irinotecan [9,10] have revealed that the VEGF-2578 AA and VEGF-1154 AA genotypes can predict better survival, whereas the VEGF-152 GG genotype indicates poorer survival. These studies only analysed MCC patients treated with chemotherapy and BEV, and did not compare the survival between patients receiving simple or combined chemotherapy; therefore, a correlation may exist between the candidate SNPs of VEGF and the efficacy and toxicity of BEV. This study investigated the correlation between the candidate SNPs of VEGF and the long-term efficacy of BEV, evaluated the pre-treatment SNPs of VEGF in 60 MCC patients, and performed long-term survival follow-up.

Materials and Methods

Clinical data

A total of 60 MCC patients treated in the First People's Hospital of Foshan from January 2012 to December 2014 were enrolled, which included 35 males and 25 females aged 33-73 y old (median 58 y) with ECOG physical status scores ≤ 2; all patients had evaluable lesions and were treated with regular first-line chemotherapy. Among these patients, 38 patients were treated with oxaliplatin-based chemotherapy (mFOLFOX6 or Xelox), and 22 were treated with FOLFIRI chemotherapy. In accordance with the corresponding chemotherapy protocols, 20 patients were treated in combination with BEV (5 mg/kg body weight, iv. gtt. once every 2 w, or 7.5 mg/kg body weight, iv. gtt. once every 3 w). Blood samples were collected from patients before chemotherapy, and all the patients were followed up closely after chemotherapy.

Design of target SNPs and primers

The primers for 10 common SNPs of VEGF were designed, and in accordance with the Mass ARRAY principle, two amplification primers were designed to amplify the target fragments. In addition, a single base extension primer was designed for each SNP, located one base before the mutation site, for extension of one base at the mutation site (Table 1). MMALDI-TOF/MS was performed to distinguish the molecular weight of the extended base so as to determine whether the target gene was mutated. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the First People's Hospital of Foshan. Written informed consent was obtained from all participants.

Detection process

Sample collection: The collected peripheral blood was centrifuged at 4000 rpm for 10 min to remove the serum; the white blood cells were collected from the buffy coat and stored at -80°C for future use.

DNA extraction and identification: The Rapid DNA extraction kit was used to extract the total DNA of white blood cells in accordance with the manufacturer's protocol. The purity and concentration of genomic DNA were quantitatively determined using a Nanodrop spectrophotometer, and the degradation of genomic DNA was observed by 2% agarose gel electrophoresis.

Mass spectrometry analysis: (1) Primers were designed using mass spectrometry software (Table 1). (2) PCR amplification reaction consisted of 0.5 μL of PCR buffer (10X), 0.4 μL of $MgCl_2$ (25 mM), 0.1 μL of dNTPs (25 mM), 0.2 μL of PCR hot start polymerase (5 U/μL), 1 μL of primer mixture, 2 μL of genomic DNA, and supplemented with water to a total volume of 5 μL. The following cycling conditions were used: 95°C for 2 min, 45 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 1 min, followed by 72°C for 5 min. (3) Shrimp Alkaline Phosphatase (SAP)-treatment: SAP was added to remove dNTPs from the PCR products. A total of 0.3 μL of SAP (1.7 U/μL) and 0.17 μL of SAP buffer (10X) (supplemented with water to 7 μL) were added into the PCR products. The reaction conditions were as follows: 37°C for 40 min and 85°C for 5 min. (4) Single base extension reaction: The following was then added to the products obtained above: 0.2 μL of iplex buffer (10X), 0.2 μL of iplex terminator, 0.041 μL of iplex enzyme, 0.94 μL of extension primer mixture, and supplemented with water to a total volume of 9 μL. The reaction conditions were as follows: 94°C for 30 s, 40 cycles of (94°C for 5 s, (52°C for 5 s and 80°C for 5 s) × 5 cycles), followed by 72°C for 3 min. (5) Resin purification: 16 μL of water was added to the products; clean resin was evenly passed into a 6 mg resin plate (96-well plate), and the resin was poured into the extension products, followed by mounting and rotating the plate for 30-60 min (deionization so as to prevent interference); the resin was then centrifuged at 3000 Xg for 5 min to allow the products to sink into the bottom of the wells. (6) Chip spotting and detection: the MassARRAY spotter was used for loading the samples, which were then analysed by the scanner. (7) Data analysis: the scan results were analysed by Typer4.0 software. Time-of-flight mass spectrometry was also performed to distinguish the mutations of the target genes according to the molecular weights of the bases at the mutation sites; the occurrence of mutations can be determined if peaks are observed at the base variation site.

Statistical analysis

SPSS16.0 software was used for the statistical analysis. The count data were analysed by the χ^2 test, and the measurement data were analysed by the mean t-test. The Kaplan-Meier method was applied for Log Rank survival analysis. $P<0.05$ was considered to indicate statistical significance.

Results

Result interpretation

The MALDI-TOF/MS figures (Figure 1) exhibit the spectrum of each target SNP. Each SNP was marked with three peaks, among which the peak with the smallest molecular weight was for the single-base extension reaction primer, and with the addition of one base into this primer, one peak appeared for a homozygote, and two peaks with relatively equal strength were considered to represent a heterozygote. The mass spectra revealed that the method was intuitive and results can be easily determined. However, if too many SNP sites were detected simultaneously, there will be some interference. When detecting rs1570360 by mass spectrometry, the spectrum revealed low product content as well as the existence of interference, and after PCR amplification, rs1570360 was hardly identified from the products in the first electrophoretic map; after changing the system and polymerase and increasing the annealing temperature, the product was finally obtained. The main reason for this observation was that rs1570360 is located in the promoter region, and the GC content is very high, which interfered with the PCR amplification. Therefore, the variations of 10 SNPs that only had the gene of this locus cannot be determined.

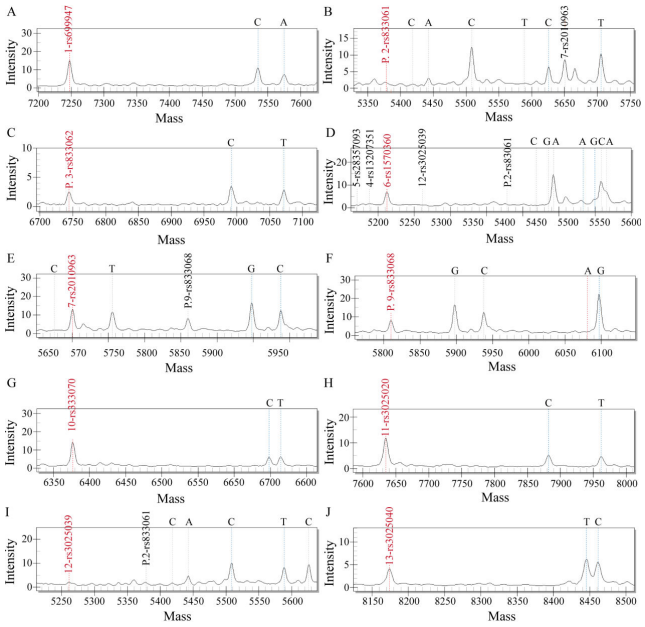

Figure 1. *Rs699947 AC heterozygote, Rs833061 TC heterozygote, Rs833062 TC heterozygote, Rs1507360, Rs2010963 GC heterozygote, Rs833068 GG homozygote, Rs833070 TC heterozygote, Rs3025020 TC heterozygote, Rs3025039 TC heterozygote and Rs3025040 TC heterozygote.*

Mutation frequency

The results show the summary and mutation frequency distribution of nine SNPs in 60 colorectal cancer samples, which was generally similar to those in the NCBI database (Table 2). Significantly, the mutations -2578C>A and -460C>T were highly consistent (59/60 98.3%), and 59 out of the 60 patients exhibited complete consistency in the homozygosis, heterozygous mutations, and homozygous mutations. The mutation status of the two SNPs in the patients treated with BEV was completely consistent.

Survival analysis

The survival status of all patients and those treated with BEV was analysed. The median OS for all patients was 16.0 months (95% CI: 9.3-22.7), and the median OS for patients treated with BEV was 34.0 months (95% CI: 95% CI: 26.5-44.4). The median OS of the single chemotherapy group was 12.7 months (95% CI: 10.8-14.6), with a statistically significant difference (P=0.01) (Figure 2A). For all patients, the OS of the patients homozygous for SNP-497TT was worse than patients with other SNPs (P=0.02) (Figure 2B). Among the patients treated with BEV, the OS of patients with the three SNP-497 genotypes were significantly different (P=0.01) (Figure 2C). The OS of patients homozygous for SNP-398AA was superior to that if patients with the other SNPs (P=0.02) (Figure 2D). The OS of patients homozygous for SNP-2455 CC was superior to that of patients with other SNPs (P=0.01) (Figure 2E).

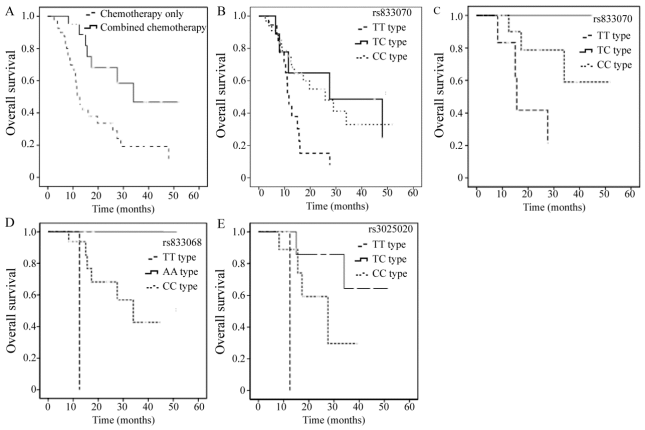

Figure 2. *A: The OS of combined therapy is superior to single chemotherapy (P=0.01); B: The OS of the patients with SNP-497TT homozygote is worse than others(P=0.01); C: There are significant differences in the OS among the three SNP-497 genotypes in the patients applied BEV(P=0.01); D: The OS of the patients with SNP-398AA homozygote is superior to others (P=0.02); E: The OS of the patients with SNP-2455 CC homozygote is superior to others (P=0.01).*

Discussion

This study included 60 MCC patients, for whom MassARRAY successfully detected the status of nine among the 10 SNPs in the *VEGFA* gene, the mutation status of which was similar to that reported in the NCBI database; the mutation rate of rs833062T>C locus was lower than 1%, which had no clinical significance; the mutations of -2578C>A and -460C>T were highly consistent with the results in the database (59/60, 98.3%), and exactly the same in the patients treated with BEV; this observation has not previously been reported, and needs further investigation. The OS analysis showed that BEV treatment significantly prolonged the survival period; among

all the patients, the OS of the patients homozygous for SNP-497TT was worse than that of patients with other SNPs (P=0.02); the OS of patients treated with BEV varied significantly depending on SNP status; those homozygous for SNP-398AAhad better survival than those with other SNPs (P=0.02), and those homozygous for SNP-2455CC had superior survival to those with other SNPs (P=0.01). One patient homozygous for-2578AAis still alive and the prognosis for this SNP appears to be the best, but no statistical difference has been found among the other patients. The results of this study are not fully consistent with those reported by other investigators. However, the survival periods were different for different SNP genotypes in the 20 patients treated with BEV, so, it will be worthwhile to increase the number of cases and SNPs examined in future studies.

The morbidity and mortality of MCC remain high, but effective drugs are limited in comparison to those available for lung cancer, breast cancer, or other diseases, cases for which have increased in number over the last 10 years. VEGF is necessary for neovascularization, and its overexpression is closely associated with the invasiveness, metastasis of vascular density, and recurrence of colorectal cancer. VEGF is an important factor in the occurrence, proliferation, and invasion of colorectal cancer cells. Patients with VEGF overexpression in malignant tumors normally have a worse prognosis. BEV is a recombinant human anti-VEGF monoclonal antibody, which can bind to various human VEGFs with high affinity, thus blocking VEGF-mediated angiogenesis and effectively inhibiting the growth and metastasis of tumor cells [11]. In 2004, Hurwitz et al. [12] first reported a phase III randomized controlled trial using the IFL regimen combined with BEV for treating MCC, where 813 enrolled patients were randomly divided into the IFL+placebo group or the IFL+BEV group. The results showed that the response rates of the two groups were 34.8% and 44.8% (P=0.004), the median OSs were 15.6 months and 20.3 months (P<0.001), and the Progress-Free Survivals (PFSs) were 6.2 months and 10.6 months, respectively (P<0.001). It can be seen that the RR, OS, and PFS in the IFL+BEV group were significantly higher than those in the control group. On February 26, 2004, the FDA approved BEV as a first-line drug against MCC, which became the first FDA-approved targeted first-line drug against MCC, through its inhibition of angiogenesis. Later, Saltz et al. [13-16] conducted a series of Phases II and III clinical studies and meta-analysis, which concluded that BEV, as the first- and second-line treatment, can improve the survival of MCC patients, so that BEV combined with chemotherapy has become the most commonly used treatment regimen for MCC in the world.

However, after more than 10 years of BEV use, there are still some researchers who that believe its performance benefits do not outweigh its risks [2]. Common side effects of BEV include hypertension, proteinuria, bleeding tendency, arterial thrombosis, or gastrointestinal perforation [17,18]; therefore, efficacy and toxicity predictors are needed to guide its rational use. A great deal of work has been done by researchers all over the world, and it is known currently that intratumoral markers such as BRAF mutation, chromosome 18 q loss of heterozygosity, RAS mutation, microsatellite instability, PIK3CA mutation, and Carcinoembryonic Antigen (CEA) are well-established predictors [19], but they can only provide prognostic information and predict the efficacy of fluorouracils and anti-EGFR. There is no known factor to predict the efficacy of BEV. Analysis of multiple tumor- and angiogenesis-associated proteins such as IL-6, IL-8, bFGF, PDGF-BB, or VEGF-A by immunohistochemistry and other tumor protein analysis methods has indicated that expression of VEGF-A/D may predict PFS [5-7]; however, relationships between the expression of other tumor proteins and ORR, PFS, or OS have not been found. Marien et al. [20] reported that anti-angiogenesis protein markers should be grouped into four groups: VEGF-signaling related proteins, other related angiogenic factors, factors related to tumor microenvironment, and intratumoral intrinsic markers; selection of the applications of angiogenesis inhibitors by appropriately combining predictive biomarkers. Therefore, the peripheral blood and tumor microenvironment may be of great value. However, initial studies in this area were unsuccessful. Pohl et al. [21] applied BEV combined with chemotherapy and examined the levels of EPCs in circulation, serum VEGF levels, and expression of VEGF in tumor tissues before and after treatment. The results showed that serum and tissue markers did not have significant predictive value. According to the study published by Liuet al. [22], patients can be divided into two groups according to the concentrations of Ang-2, E-Cadherin, IL-6, MCP-1, OPN, and TGF-β1 in the peripheral blood, namely, with poor prognosis and good prognosis, and consequently, studies looking at serum markers may be of some value. Willett et al. [8] published a study in 2009 that investigated the predictive effects of the changes of blood VEGF concentration, and no study could obtain similar results until Azzariti et al. [23] reported that the ratio of BEV-free VEGF, total VEGF serum, and plasma BEV concentration can be used as promising biomarker for predicting the responses to BEV combined with oxaliplatin.

There is no simple method for finding predictors in the peripheral blood. Detection of VEGF-SNPs in the peripheral blood is simple, and may be related to the ability of BEV to bind to VEGF. It can predict the level of free VEGF, thus exhibiting important research value. However, as for its value in predicting response to BEV treatment, although certain studies reported positive results [9,10], the real value is still controversial. Subsequent results have not been consistent, and Hansen et al. [24] considered that presence of the VEGFR-1 319 AA mutation can suggest the best short-term response to BEV treatment combined with chemotherapy, but other SNPs had no predictive value; Loupakis et al. [25] published a prospective study that detected main SNPs of VEGF/VEGFR, but was not able to verify the finding that the VEGFA rs833061 SNP can affect prognosis. Only the VEGFR2 rs12505758 SNP was associated with patient PFS. A recent study [26] found that the VEGFR rs833061 SNP was associated with ORR, and FLT1 rs9513070 SNP and FLT1 GCA haplotype were associated with PFS and OS in patients

with advanced colorectal cancer treated with chemotherapy combined with BEV. In this study, SNPs with prognostic significance were rs833068, rs833070, andrs3025020. This is different from the abovementioned reports from the Caucasian population. It is possible that VEGF-SNPs in Chinese are different from that in the Western population. Of course, lack of sample size in the present study may also have led to the above differences.

Although the number of cases in this study was small, the follow-up time was fairly long, so it can still reveal potential prognostic values of multiple VEGF SNPs, and it is worthy of further research. We have begun to prospectively sample blood specimens from the patients treated with BEV and increased the number of research centers. Furthermore, dynamic serum VEGF monitoring will be carried out when conditions permit in the future, hoping to gain predictive values of BEV efficacy as early as possible, with regard to the SNPs in the VEGF/VEGFR pathway.

In summary, the VEGF/VEGFR pathway is an important research topic for determining BEV's efficacy prediction among intratumoral markers and other sample types, including peripheral blood. Although the predictive value of each SNP is still controversial, it is worthy of studies and explorations owing to the lack of such research in the Chinese population.

Although the number of cases in this study is small, the follow-up time is longer, so it can still reveal potential prognostic values of multiple VEGF SNPs, and it's worth further research. We have begun to prospectively sample blood specimens from the patients treated with BEV and increased the number of research centers; furthermore, dynamic serum VEGF monitoring will be carried out when conditions permit in the future, hoping to gain predictive values of BEV efficacy as early as possible concerning the SNPs in the VEGF/VEGFR pathway.

Table 1. *MassARRAY primers and probes.*

ID	SNP	PCR-F	PCR-R	Single base extension primer
rs699947	-2578 C>A	ACGTTGGATGGCATATAGGAAGCAGCTTGG	ACGTTGGATGTTCCCATTCTCAGTCCATGC	TCAGTCTGATTATCCACCCAGATC
rs833061	-460 T>C	ACGTTGGATGTGAGTGAGTGTGTGCGTGTG	ACGTTGGATGAGGAAAGTGAGGTTACGTGC	GCGTGTGGGGTTGAGGG
rs833062	-1455 T>C	ACGTTGGATGTGAGTGAGTGTGTGCGTGTG	ACGTTGGATGAGGAAAGTGAGGTTACGTGC	AGGGGTCACTCCAGGATTCCAA
rs1570360	-1154 G>A	ACGTTGGATGCCCAAAAGCAGGTCACTCAC	ACGTTGGATGGCTCTACTTCCCCAAATCAC	CACTCACTTTGCCCCTGTC
rs2010963	-634 G>C	ACGTTGGATGCCCAAAAGCAGGTCACTCAC	ACGTTGGATGGCTCTACTTCCCCAAATCAC	CACTCACTTTGCCCCTGTC
rs833068	-398 G>A	ACGTTGGATGAGCAGGAAGACAGTGTTCAG	ACGTTGGATGAGAGATCCCATTAGGCTGAG	TCCCATTTGTGGGAACTGT
rs833070	-497 T>C	ACGTTGGATGACAACGGAACAAAAGGCAGG	ACGTTGGATGTCAGCCTAATGGGATCTCTC	ACAGCACCCGAACATAGTCAA
rs3025020	-2455>C-T	ACGTTGGATGTTCATCTGGTGGAAGTGCCC	ACGTTGGATGAAAGTAGGGTGTGATGGGAG	GCCTCTGGAGGGGAGCCCCCTATTC
rs3025039	-936 C>T	ACGTTGGATGCATCACCATCGACAGAACAG	ACGTTGGATGCTCGGTGATTTAGCAGCAAG	GGGCGGGTGACCCAGCA
rs3025040	-752 C>T	ACGTTGGATGCAGAAGCAGGTGAGAGTAAG	ACGTTGGATGAGACAGATCACAGGTACAGG	AAGCTCCCCAAACTCCTGGTCAGAGCC

Table 2. *Summary of SNPs in 60 colorectal cancer samples by MassARRAY.*

n	rs699947 C>A	rs833061 T>C	rs833062 T>C	rs302503 C>T	rs833068G>A	rs2010963G>C	rs833070 C>T	rs3025020C>T	rs3025040C>T
Homozygosis of original gene	32	33	59	41	53	21	33	28	39
Heterozygous mutation	22	21	1	19	1	31	9	29	21
Homozygous mutation	6	6	0	0	6	8	18	3	0
Mutation frequency	A=0.2833	C=0.275	C=0.0083	T=0.1583	A=0.1083	C=0.3917	T=0.375	T=0.2917	T=0.175
Mutation frequency in NCBI	A=0.3245	C=0.3698	C=0.0050	T=0.1336	A=0.3814	C=0.3261	T=0.3393	T=0.2344	T=0.1512

Acknowledgements

This study was supported by the Projects of Foshan Science and Technology Bureau (2014AB00278), Special Funds of Foshan Science and Technology Innovation (2014AG10003) and Projects of Wu Jieping Foundation (320.6700.1143).

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
2. Hoyle M, Crathorne L, Peters J, Jones-Hughes T, Cooper C, Napier M, Tappenden P, Hyde C. The clinical effectiveness and cost-effectiveness of cetuximab (mono- or combination chemotherapy), bevacizumab (combination with non-oxaliplatin chemotherapy) and panitumumab (monotherapy) for the treatment of metastatic colorectal cancer after first-line chemotherapy (review of technology appraisal No.150 and part review of technology appraisal No. 118): a systematic review and economic model. Health Technol Assess 2013; 17: 1-237.
3. Wang W, Zhao LR, Lin XQ, Feng F. Reversible posterior leukoencephalopathy syndrome induced by bevacizumab plus chemotherapy in colorectal cancer. World J Gastroenterol 2014; 20: 6691-6697.
4. Luo HY, Xu RH. Predictive and prognostic biomarkers with therapeutic targets in advanced colorectal cancer. World J Gastroenterol 2014; 20: 3858-3874.
5. Sclafani F, Rimassa L, Colombo P, Destro A, Stinco S, Lutman FR, Carnaghi C, Beretta G, Zanello A, Roncalli M, Giordano L, Santoro A. An exploratory biomarker study in metastatic tumors from colorectal cancer patients treated with bevacizumab. Int J Biol Markers 2015; 30: 73-80.
6. Bruhn MA, Townsend AR, Khoon Lee C, Shivasami A, Price TJ. Proangiogenic tumor proteins as potential predictive or prognostic biomarkers for bevacizumab therapy in metastatic colorectal cancer. Int J Cancer 2014; 135: 731-741.
7. Weickhardt AJ, Williams DS, Lee CK, Chionh F, Simes J, Murone C, Wilson K, Parry MM, Asadi K, Scott AM, WilsonK, Parry MM, Asadi K, Scott AM, Punt CJA, Nagtegaal ID, Price TJ, Mariadason JM, Tebbutt NC. Vascular endothelial growth factor D expression is a potential biomarker of bevacizumab benefit in colorectal cancer. Br J Cancer 2015; 113: 37-45.
8. Willett CG, Duda DG, di Tomaso E, Boucher Y, Ancukiewicz M, Sahani DV, Lahdenranta J, Chung DC, Fischman AJ, Lauwers GY, Shellito P, Czito BG, Wong TZ, Paulson E, Poleski M, Vujaskovic Z, Bentley R, Chen HX, Clark JW, Jain RK. Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: a multidisciplinary phase II study. J Clin Oncol 2009; 27: 3020-3026.
9. Formica V, Palmirotta R, Del Monte G, Savonarola A, Ludovici G, De Marchis ML, Grenga I, Schirru M, Guadagni F, Roselli M. Predictive value of VEGF gene polymorphisms for metastatic colorectal cancer patients receiving first-line treatment including fluorouracil, irinotecan, and bevacizumab. Int J Colorectal Dis 2011; 26: 143-151.
10. Napoleone F. Vascular endothelial growth factor as a target for anticancer therapy. Oncologist 2004; 9: 2-10.
11. Koutras AK, Antonacopoulou AG, Eleftheraki AG, Dimitrakopoulos FI, Koumarianou A, Varthalitis I, Fostira F, Sgouros J, Briasoulis E, Bournakis E, Bafaloukos D, Bompolaki I, Galani E, Kalogeras KT, Pectasides D, Fountzilas G, Kalofonos HP. Vascular endothelial growth factor polymorphisms and clinical outcome in colorectal cancer patients treated with irinotecan-based chemotherapy and bevacizumab. Pharmacogenomics J 2012; 12: 468-475.
12. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004; 350: 2335-2342.
13. Kopetz S, Glover KY, Eng C,Wolff RA, Chang DZ, Adinin RB, Morris J, Abbruzzese JL, Hoff PM. Phase II study of infusional 5-fluorouracil, leucovorin, and irinotecan (FOL FIRI) plus bevacizumab as first-line treatment for metastatic colorectal cancer. J Clin Oncol 2007; 25: 4089.
14. Saltz LB, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzén F, Cassidy J. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol 2008; 26: 2013-2019.
15. Giantonio BJ, Catalano PJ, Meropol NJ,ODwyer PJ, Mitchell EP, Alberts SR, Schwartz MA, Benson AB. Eastern Cooperative Oncology Group Study E3200: Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol 2007; 25: 1539-1544.
16. Ilic I, Jankovic S, Ilic M. Bevacizumab combined with chemotherapy improves survival for patients with metastatic colorectal cancer: evidence from meta-analysis. PLoS One 2016; 11: 0161912.
17. Kiss I, Bortlicek Z, Melichar B, Poprach A, Halamkova J, Vyzula R, Dusek L, Buchler T. Efficacy and toxicity of bevacizumab on combination with chemotherapy in different lines of treatment for metastatic colorectal carcinoma. Anticancer Res 2014; 34: 949-954.
18. Wagner AD, Arnold D, Grothey AA, Haerting J, Unverzagt S. Anti-angiogenic therapies for metastatic colorectal cancer. Cochrane Database Syst Rev 2009; 8: 5392.
19. Lech G, Slotwinski R, Slodkowski M, Krasnodebski IW. Colorectal cancer tumour markers and biomarkers: Recent

therapeutic advances. World J Gastroenterol 2016; 22: 1745-1755.

20. Marien KM, Croons V, Martinet W, De Loof H, Ung C, Waelput W, Scherer SJ, Kockx MM, De Meyer GR. Predictive tissue biomarkers for bevacizumab-containing therapy in metastatic colorectal cancer: an update. Expert Rev Mol Diagn 2015; 15: 399-414.
21. Pohl M, Werner N, Munding J, Tannapfel A, Graeven U, Nickenig G, Schmiegel W,Reinacher-Schick A. Biomarkers of anti-angiogenic therapy in metastatic colorectal cancer (mCRC): original data and review of the literature. Z Gastroenterol 2011; 49: 1398-1406.
22. Liu Y, Starr MD, Bulusu A, Pang H, Wong NS, Honeycutt W, Amara A, Hurwitz HI, Nixon AB. Correlation of angiogenic biomarker signatures with clinical outcomes in metastatic colorectal cancer patients receiving capecitabine, oxaliplatin, and bevacizumab. Cancer Med 2013; 2: 234-242.
23. Azzariti A, Porcelli L, Brunetti O, Pang H, Wong NS, Honeycutt W, Amara A, Hurwitz HI, Nixon AB. Total and not bevacizumab-bound vascular endothelial growth factor as potential predictive factors to bevacizumab-based chemotherapy in colorectal cancer. World J Gastroenterol 2016; 22: 6287-6295.
24. Hansen TF, Christensen RD, Andersen RF, Garm Spindler KL, Johnsson A, Jakobsen A. The predictive value of single nucleotide polymorphisms in the VEGF system to the efficacy of first-line treatment with bevacizumab plus chemotherapy in patients with metastatic colorectal cancer: results from the Nordic ACT trial. Int J Colorectal Dis 2012; 27: 715-720.
25. Loupakis F, Cremolini C, Yang D, Salvatore L, Zhang W, Wakatsuki T, Bohanes P, Schirripa M, Benhaim L, Lonardi S, Antoniotti C, Aprile G, Graziano F, Ruzzo A, Lucchesi S, Ronzoni M, De Vita F, Tonini G, Falcone A, Lenz HJ. Prospective validation of candidate SNPs of VEGF/VEGFR pathway in metastatic colorectal cancer patients treated with first-line FOLFIRI plus bevacizumab. PLoS One 2013; 8: 66774.
26. Sohn BS, Park SJ, Kim JE, Kim KP, Hong YS, Suh C, Kim YS, Kim SY, Im SA, Kim SY, Kim JH, Ahn JB, Park YS, Kim TW. Single-nucleotide polymorphisms in the vascular endothelial growth factor pathway and outcomes of patients treated with first-line cytotoxic chemotherapy combined with bevacizumab for advanced colorectal cancer. Oncology 2014; 87: 280-292.

*Correspondence to

Wei Wang

Cancer Center

The First People's Hospital of Foshan

PR China